

CLAIMS:

1. A method of producing isolated IgG1 subclass antibodies reactive to the surface of *Cryptosporidium* oocysts, the method comprising:
  - (a) pretreating *Cryptosporidium* oocysts with a reagent so as to remove the surface layer of the oocysts to form an oocyst antigen preparation;
  - (b) separating the oocysts from the oocyst antigen preparation so as to obtain a separated oocyst antigen preparation capable of eliciting a detectable IgG1 immune response in an animal to the surface of the oocyst;
  - (c) immunising an animal with the separated oocyst antigen preparation so as to elicit an IgG1 immune response in the animal; and
  - (d) obtaining from the animal IgG1 antibodies reactive to the surface of *Cryptosporidium* oocysts.
2. The method according to claim 1 wherein the reagent is a detergent.
3. The method according to claim 2 wherein the detergent is sodium dodecyl sulphate (SDS).
4. The method according to claim 3 wherein the pretreating is boiling the oocysts in the presence of SDS for a sufficient time to generate the oocyst antigen preparation.
5. The method according to claim 4 wherein (a) is boiling the oocysts for 1 hour in the presence of 0.5% (w/v) SDS.
6. The method according to claim 1 wherein the reagent is selected from the group consisting of urea, detergents including Triton X-100 and nonident, enzymes including chitinase, oxidising agents including sodium hypochlorite, sodium periodate, and ozone; and reducing agents including mercaptol ethanol and 1,1,1-trichloro- 2,2-bis[4-chlorophenyl]ethane.
7. The method according to any one of claims 1 to 6 wherein (c) further includes one or more adjuvants.
8. The method according to any one of claims 1 to 7 wherein the animal is a mouse.
9. A method of producing isolated IgG1 subclass antibodies reactive to the surface of *Cryptosporidium* oocysts, the method comprising:
  - (a) separating at least a portion of the *Cryptosporidium* oocyst wall from the internal sporozoites to form an oocyst-wall preparation;
  - (b) treating the separated oocyst-wall preparation so as to obtain an oocyst antigen preparation capable of eliciting a detectable IgG1 immune response in an animal to the surface of the oocyst;

CLAIMS:

1. A method of producing isolated IgG1 subclass antibodies reactive to the surface of *Cryptosporidium* oocysts, the method comprising:  
(a) pretreating *Cryptosporidium* oocysts with a reagent so as to remove the surface layer of the oocysts to form an oocyst antigen preparation;  
(b) separating the oocysts from the oocyst antigen preparation so as to obtain a separated oocyst antigen preparation capable of eliciting a detectable IgG1 immune response in an animal to the surface of the oocyst;  
(c) immunising an animal with the separated oocyst antigen preparation so as to elicit an IgG1 immune response in the animal; and  
(d) obtaining from the animal IgG1 antibodies reactive to the surface of *Cryptosporidium* oocysts.

2. The method according to claim 1 wherein the reagent is a detergent.

3. The method according to claim 2 wherein the detergent is sodium dodecyl sulphate (SDS).

4. The method according to claim 3 wherein the pretreating <sup>comprises</sup> is boiling the oocysts in the presence of SDS for a sufficient time to generate the oocyst antigen preparation.

5. The method according to claim 4 wherein <sup>the boiling of</sup> (a) is boiling the oocysts for 1 hour in the presence of 0.5% (w/v) SDS.

6. The method according to claim 1 wherein the reagent is selected from the group consisting of urea, detergents, including Triton X-100 and nonident, enzymes, including chitinase, oxidising agents including sodium hypochlorite, sodium periodate, and ozone; and reducing agents including mercaptol ethanol and 1,1,1-trichloro- 2,2-bis[4-chlorophenyl]ethane.

7. The method according to <sup>Claim 1</sup> ~~any one of claims 1 to 6~~ wherein <sup>the preparation of step</sup> (c) further includes one or more adjuvants.

8. The method according to <sup>Claim 1</sup> ~~any one of claims 1 to 7~~ wherein the animal is a mouse.

9. A method of producing isolated IgG1 subclass antibodies reactive to the surface of *Cryptosporidium* oocysts, the method comprising:

(a) separating at least a portion of the *Cryptosporidium* oocyst wall from the internal sporozoites to form an oocyst-wall preparation;  
(b) treating the separated oocyst-wall preparation so as to obtain an oocyst antigen preparation capable of eliciting a detectable IgG1 immune response in an animal to the surface of the oocyst;

(c) immunising an animal with the oocyst antigen preparation so as to elicit an IgG1 immune response in the animal; and

(d) obtaining from the animal IgG1 antibodies reactive to the surface of *Cryptosporidium* oocysts.

5 10. The method according to claim 9 wherein the separation of the oocyst wall from the internal sporozoites <sup>comprises inducing</sup> ~~is by causing~~ the oocyst to excyst followed by immuno-separation of the oocyst wall components.

10 11. The method according to claim 9 wherein the separation of the oocyst wall from the internal sporozoite <sup>comprises inducing</sup> ~~is by causing~~ the oocyst to excyst followed by separation of the wall components by the group consisting of centrifugation, <sup>means selected from</sup> flow cytometry, density gradient separation, precipitation, immuno-labelling, ligand-binding, biotin-labelling <sup>with</sup> ~~and~~ separation by avidin, and chromatographic separation.

15 12. The method according to claim 10 <sup>comprises</sup> ~~or 11~~ wherein <sup>inducing</sup> ~~causing~~ the oocyst to excyst <sup>the oocyst</sup> ~~is by~~ freeze-thawing or by physically breaking up by crushing, sonication, or grinding.

20 13. The method according to <sup>claim 9</sup> ~~any one of claims 9 to 12~~ wherein the treating step (b) <sup>comprises</sup> ~~is by~~ physically breaking up the cell wall.

25 14. The method according to <sup>claim 9</sup> ~~any one of claims 9 to 13~~ wherein (c) further includes one or more adjuvants. <sup>the preparation of step</sup>

30 15. The method according to <sup>claim 9</sup> ~~any one of claims 9 to 15~~ wherein the animal is a mouse.

35 16. An isolated IgG1 antibody reactive to the surface of *Cryptosporidium* oocysts produced by the method according to <sup>claim 1</sup> ~~any one of claims 1 to 8~~.

17. The antibody according to claim 16 <sup>wherein the antibody is</sup> ~~being~~ a monoclonal antibody.

18. An isolated IgG1 antibody reactive to the surface of *Cryptosporidium* oocysts produced by the method according to <sup>claim 9</sup> ~~any one of claims 9 to 15~~.

19. The antibody according to claim 18 <sup>wherein the antibody is</sup> ~~being~~ a monoclonal antibody.

20 20. An isolated IgG1 antibody reactive to the surface of *Cryptosporidium* oocysts, <sup>wherein</sup> ~~the antibody~~ <sup>has</sup> ~~having~~ the oocyst binding and affinity characteristics of antibody CRY104.

21. The antibody according to claim 20 <sup>wherein</sup> ~~being~~ a monoclonal antibody.

22. The antibody according to claim 21 <sup>is</sup> ~~being~~ the IgG1 monoclonal antibody produced by clone CRY104. <sup>hybridoma</sup>

23. The hybridoma clone CRY104.

Wtc  
D5

0544046-022400